REMARKS

Reconsideration and allowance of this application are respectfully requested.

The claims have been amended to specify that the bacterium is an --*Escherichia coli*-- bacterium. Consequently, claims 2-4 have been cancelled. Support for the amendment is found, inter alia, at page 5, line 5 to page 6, line 19 of the specification and in the Examples on pages 12-33 of the specification.

Claim 17, which is directed to a deposited strain (strain PTL003) described in the Examples in the specification, has been added.

Claim 1, 5-12 and 16-17 are pending.

Restriction Requirement

The Examiner made the restriction requirement final. The claim of non-elected Group III (claim 14) has been cancelled. However, claims of the other two non-elected groups, Groups II and IV, have been retained because Applicant believes that the Examiner will be able to rejoin the claims of those two non-elected groups with elected claims 1-11 after their allowance. In this regard, Applicant asks the Examiner to note that the remaining claims of non-elected Groups II and IV (claims 12 and 16) depend from claim 1 and that in fact all the claims in the application incorporate the subject matter of claim 1. Therefore, if the Examiner finds the subject matter of claim 1 to be patentable, the same arguments for patentability apply to all claims sharing the same inventive concept.

Rejection under 35 USC § 112

The Examiner suggested that the Applicant should make a deposit of *E. coli* strain E1392/75/2A. This is the starting strain used in the experiments described in the Examples of the specification. Applicant introduced mutations into the *aroC*, *ompF*, and *ompC* genes of this strain to produce a strain according to the invention, which is called strain PTL003.

Thus, Applicant believes that strain PTL003 is a more appropriate strain to deposit than strain E1392/75/2A and, accordingly, wishes to rely on a deposit of the former strain rather than of the latter. Applicant has amended the specification to recite the details of the deposit. This deposit was made under the provisions of the Budapest Treaty and has been accepted by the International Depository Authority. All restrictions upon public access to the deposit will be irrevocably removed upon the grant of a patent on this application.

Rejections under 35 USC § 103

The Examiner rejected claims 1-8, 10-11 and 13 as being unpatentable over Charles et al. (U.S. Patent No. 5,683,700) in view of Chatfield et al. Reconsideration of the rejection is requested in light of the claim amendments and the following submissions.

In order to be useful in a vaccine, a live attenuated bacterium must have a good balance of the following two properties:

(i) It must be sufficiently immunogenic that it generates a potent immune response against the pathogenic form of the bacterium.

(ii) It must be sufficiently attenuated that it does not cause the disease caused by the pathogenic form of the bacterium.

The present bacteria have a surprisingly good balance of these properties. They cause no statistically significant disease symptoms beyond placebo whatsoever and yet are highly immunogenic. They are more immunogenic than *ompR aroC* mutants which the Examiner argues are disclosed in Charles et al.

As evidence of this, a Declaration from Dr Michael Darsley is attached. The Declaration describes two clinical trials. As Dr Darsley explains, in both trials, two strains of bacteria were tested, strain "PTL-ETEC-002" and strain "PTL-ETEC-003". Strain PTL-ETEC-002 is attenuated by mutations in the *ompR* and *aroC* genes, and is therefore a strain within the Examiner's interpretation of Charles et al. PTL-ETEC-003 is attenuated by mutations in *aroC*, *ompC* and *ompF* and is a strain according to the present invention.

The first trial showed that both the *aroC ompR* and *aroC ompC ompF* mutant strains were safe and immunogenic.

The enlarged second trial was designed specifically to detect differences between the two strains. The overall incidences of general symptoms of side-effects were not significantly different for subjects receiving either test strain compared to placebo recipients. However, the *aroC ompC ompF* strain according the invention was superior from the point of view of inducing an immune response. See the "Discussion and Overall Conclusions" section on page 41 of the report of the second study (Exhibit 2 to Dr Darsley's Declaration), where it is stated that:

> "The PTL-ETEC-003 construct was superior to the PTL-ETEC-002 construct in its ability to induce both mucosal and systemic immune responses to CFA/II. PTL-

ETEC-003 also exhibited a more sustained intestinal colonization than PTL-ETEC-002."

Applicant respectfully submits that the combination of references cited by the Examiner, Charles et al. and Chatfield et al., would not have made it obvious to the skilled person to consider making the bacteria recited in the claims and would not have made it obvious that the bacteria have the superior balance of properties described above. The claims now require a specific combination of elements; they require a specific species of bacterium, *E. coli*, and mutations in three specific genes of that species, namely the *aroC*, *ompF* and *ompC* genes. In order to arrive at this combination, the skilled person would have had to pick and choose from a vast number of alternatives that might have been open to him. In particular, the skilled person would have to have:

- chosen to combine the Charles et al. patent with the Chatfield et al. paper out of all the other combinations of documents that he could have made from the thousands of documents available in the art of attenuated bacterial vaccines; and

- chosen *Escherichia coli* as a species to work on, despite the fact that Charles et al. and Chatfield et al. are both very much focused on *Salmonella typhimurium*; and

- chosen to focus on mutations in *aroC* and *ompR* out of the large number of attenuating mutations that are encompassed by the disclosure of Charles et al.; and

- chosen to replace the *ompR* mutations according to Charles et al. with *ompC* and *ompF* mutations or chosen to add *ompC* and *ompF* mutations to a single *aroC* mutation according to Charles et al.

Applicant respectfully submits that this combination of four choices was not obvious. Applicant will comment further on each choice below.

The first choice that the skilled person would have had to have made, i.e., combining Charles et al. with Chatfield et al., raises the issue that the test for obviousness over a combination of references should involve a consideration of whether it was obvious to make the combination in the first place. The USPTO's Manual of Patent Examining Procedure (M.P.E.P.) repeatedly makes this clear. For example, the Manual states that:

> "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (M.P.E.P. 2143.01).

"The combination of the references taught every element of the claimed invention, however without a motivation to combine, a rejection based on a prima facie case of obviousness was held improper" (M.P.E.P. 2143.01).

Thus, Applicant respectfully submits that the Examiner should bear in mind that her rejection relies on combining a relatively obscure U.S. patent, Charles et al., with a relatively obscure literature reference, Chatfield et al. There is no clear reason why a skilled person would have picked and chosen these two documents out of the thousands of documents available in the art and chosen to combine them together. Even if a skilled person had for some reason given serious consideration to Charles et al., Charles et al. did not provide any motivation to combine its teaching with Chatfield et al. Charles et al. did not suggest replacing an *ompR* mutation with *ompC* and *ompF* mutations, and certainly did not suggest that this would lead to a highly effective vaccine. Thus, there was insufficient motivation for a skilled person who had read Charles et al. to then combine it with Chatfield et al.

The second choice that a skilled person would have to have made to arrive at the invention was to choose *Escherichia coli* as the species to work on. It seems unlikely that a skilled person would have made this choice in view of the fact that Charles et al. and Chatfield et al. are both very much focussed on *Salmonella typhimurium*. Charles et al does mention *E. coli*, but that is only in the context of a laundry list of bacterial species at column 2, lines 18-30. *S. typhimurium* and *E. coli* are very different bacteria. *S. typhimurium* is used as the basis of mouse model for human typhoid. *E. coli* causes fundamentally different diseases such as diarrhea.

The third choice that the skilled person would have to have made was to focus on mutations in *aroC* and *ompR* out of the large number of combinations of attenuating mutations that are encompassed by the disclosure of Charles et al. The disclosure of Charles et al. encompasses mutations in any of the ten genes involved in the synthesis of chorismate (e.g., *aro A*, *aroC*, *aro D* and *aroE*), *ompR* or any of "a large number of other genes which are concerned with regulation and are known to respond to environmental stimuli", and genes that produce proteins in response to environmental stress (e.g., htrA, grpE, groEL, dnaK, groES, lon and dnaJ); see column 2, line 31 to column 3, line 54. There is no particular reason why a skilled person would have focussed on the combination of *aroC* and *ompR* out of all these possibilities. The Examples of Charles et al. describe *aroA aroD* mutants.

The fourth choice that a skilled person would have to have made to arrive at the invention was to replace the *ompR* mutations according to Charles et al. with *ompC* and *ompF* mutations or chosen to add *ompC* and *ompF* mutations to a single *aroC* mutation according to Charles et al. This choice also contributes to the non-obviousness of the invention. Charles et al. provides no motivation whatsoever to replace an *ompR* mutation with *ompC* and ompF mutations. On the contrary, the only alternatives to *ompR* mutations taught in Charles et al. are mutations in "another gene involved in regulation" (column 2, lines 53-60). The *OmpC* and *OmpF* genes are not involved in regulation but rather encode structural proteins, in particular porins.

Applicant respectfully submits that there was no information available in the art which would have suggested to a skilled person that making the above series of choices would be especially beneficial in respect of producing a vaccine. The superior effectiveness of a vaccine obtained by making the choices, as demonstrated in the attached Declaration of Dr. Darsley, was not predictable. Accordingly, the claimed invention was not obvious.

The Examiner also rejected claims 1-5, 7-11 and 13 under 35 USC 103 as allegedly being unpatentable over Charles et al. (WO 92/15689) in view of the same Chatfield et al. reference as discussed above. Charles et al. (WO 92/15689) is the PCT application from which the U.S. patent of Charles et al. discussed above (U.S. Patent No. 5,683,700) is derived. Thus, Applicant believes that the rejection over Charles et al. (WO 92/15689) in view of Chatfield et al. should be withdrawn for the same reasons as set forth above in response to the rejection over Charles et al. (U.S. Patent No. 5,683,700) in view of Chatfield et al.

Information Disclosure Statement

Applicant asks the Examiner kindly to initial and return the Information Disclosure Statement filed on December 4, 2000.

It is respectfully submitted that the application is in condition for allowance, and a notice to that effect is requested.

Respectfully submitted,
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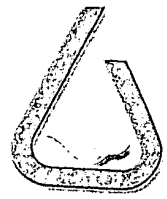

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03 September 2001

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